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INFORMATION ON THE BOOK "USSR WORK ON THE BIOCHEMICAL ASPECTS
OF MEDICAL BACTERIOLOGY" BY V. S. GOSTEV.

The following information was taken from the preface, introduction, 12 of the 13 chapters, conclusion, and bibliography of the book Biochimicheskiye Osnovy Meditsinskoy Bakteriologii, by V. S. Gostev, published by the Publishing House of the Academy of Medical Sciences USSR, 1951. The table of contents is also presented.

Information in the text of the book which appears to be of greatest interest or discusses new and unusual results reported by Soviet investigators is included.7

TABLE OF CONTENTS

	<u>Page</u>
Preface	3
Introduction	5
I. Water Content of Bacteria	7
II. Inorganic Components of Bacteria	8
III. Bacterial Proteins	11
IV. Nucleoproteids of Bacteria	14
V. Bacterial polysaccharides	25

- 1 -

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	<u>Page</u>
VI. Lipids of Bacteria	35
VII. Complete Bacterial Antigen	40
VIII. Physicochemical Properties of Bacteria	47
IX. Theory of Staining of Bacteria	58
X. Vitamins of Bacteria	70
XI. Carbohydrate Metabolism of Bacteria	96
XII. Protein Metabolism of Bacteria	118
XIII. Respiration of Bacteria	133
Conclusion	155
Bibliography	159

PREFACE

The present book represents the results of 15 years of work by the author in collecting material which characterizes the biochemical aspects of the living processes of pathogenic bacteria. A systematic exposition of the subjects was given by the author in a course of lectures held by him during 1936 - 1938 at the Chair of Bacteriology, Central Institute of Advanced Training for Physicians. On the basis of these lectures, the chapters on morphology and physiology of bacteria for the textbook of medical microbiology by Muir and Ritchie, which appeared in a Russian translation in 1938 (translator, V. S. Gostev), were completely rewritten. At present, the material on microbiological chemistry used in 1938 is no longer valid. The author revised and supplemented the original subject matter thoroughly in connection with a course of lectures held by him in 1949 at the Institute of Experimental Biology, Academy of Sciences USSR, for aspirants-immunologists of Moscow. These lectures form the basis of the present book.

The book is written for the benefit of rank-and-file bacteriologists, i.e., for readers who have a very thorough knowledge of bacteriology and are familiar with the fundamentals of biochemistry. Only the general outlines of the subject are given: no attempt at a complete treatment was made. Work done by Russian investigators was emphasized to establish the priority of national science in solving a number of problems in the fields of general biochemistry and bacteriology.

A unified treatment from the standpoint of Michurin and Lysenko's theory was given to the material presented in the book.

INTRODUCTION

Bacteria differ from other living beings in that their chemical composition varies depending on the nutrient medium and the temperature at which they have been cultivated.

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I. WATER CONTENT OF BACTERIA

[This chapter did not contain information of sufficient interest to be included here.]

II. INORGANIC COMPONENTS OF BACTERIA

In preparing synthetic nutrient media, K, Mg, and Ca salts must be used besides NaCl. A solution of NaCl without the addition of salts of divalent cations is toxic to bacteria, although they can stand higher concentrations of NaCl than other animal and plant cells.

Trace elements are essential to bacteria. This includes Fe, Zn, Mn, and Cu as well as Co. The last-named element is necessary for the activity of Vit B₁₂, into whose composition it enters.

III. BACTERIAL PROTEINS

Work on the aminoacid composition of bacterial proteins has been done by A. N. Belozerskiy, T. N. Evreinova (1948), and O. P. Samarina et al (1950).

The process of the synthesis of aminoacids and proteins from alpha-ketoacids and ammonium salts was originally subjected to detailed investigation by a number of USSR scientists. Ye. D. Vyshepan and Z. G. Mogilevskaya (1948; Vopr. Med. Khimii Vol I, No 1-2, pp 255-275, 1949) demonstrated in experiments with *B. coli* that in the presence of ammonium carbonate, on the one hand, and pyruvic acid, glucose, alpha-ketoglutaric acid, or oxaloacetic acid, on the other hand, considerable quantities of ammonia are chemically combined. The total ammonia nitrogen which has disappeared can be detected in the trichloroacetic acid precipitate from the bacteria, having entered into the composition of their proteins. A. S. Konikova, M. G. Kritsman, and L. M. Yakobson, in a number of investigations published in 1948 - 1949 (cf. A. S. Konikova, II Sessiya Otd. Med. Biol. Nauk, Akad Med Nauk SSSR; Problema Belka /Second Session, Department of Medical and Biological Sciences, Academy of Medical Sciences USSR, The Protein Problem⁷, p 25, 1949) paid particular attention to the formation of amino acids by various bacteria in a medium containing ammonium salts and various keto acids having an asymmetric alpha-carbon atom (pyruvic, phenyl pyruvic, alpha-ketoglutaric, malic, ketoedipic, and glyoxylic acids). Conducting investigations on hay bacilli, *B. brevis*, *B. coli*, *Staph. aureus*, diphtheria bacilli, typhoid bacilli, dysentery bacilli, cholera vibrios, and pseudocholera vibrios, the authors, using various methods, demonstrated the synthesis of amino acids by these microbes. It was shown that the bacterial enzymes which catalyze this synthesis are rigidly specific with regard to the substrates on which they act and that they differ from each other with regard to a number of properties. At present, one may consider as proven that bacterial cells require for their living processes the presence in the nutrient medium of a source of nitrogen, as well as of an organic compound which is structurally analogous to levo-rotatory lactic acid. The glucose which is added to a synthetic nutrient medium, or some other source of carbon which is present, serves not only as a source of energy to the multiplying bacteria but also as a material from which their specific proteins are synthesized.

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IV. NUCLEOPROTEIDS OF BACTERIA

In recent years, A. N. Belozerskiy demonstrated that the protein part of nucleoprotein molecules does not consist exclusively of basic proteins (proteins and histone), as was formerly assumed, but that complex proteins exhibiting a much wider range of amino-acid composition are involved. Such proteins are bound to nucleic acids through their amino groups (A. N. Belozerskiy and G. Bazhulina, 1944).

The complex problem of bacterial nuclei consisting of nucleoproteids which contain deoxyribonucleic acids and give Feulgen's nuclear reaction) was solved mainly thanks to work done by USSR investigators. A. A. Imshenitskiy (1948) proved that the nuclei described earlier by various authors were actually artefacts. He established (1935, 1940) that while bacteria are able to synthesize the deoxyribonucleic acid which they always contain, no visible chromatin structures that can be regarded as nuclei are present. The nuclei, while differentiated chemically, do not form distinct morphological structures; they are dispersed throughout the cell. A morphologically differentiated chromatin structure representing a nucleus forms only when the bacterium enters a state of rest, e.g., during spore formation. As long as a nucleus of this type is present, the bacterial cell is unable to propagate by division. Separation of nuclear chromatin also occurs when bacteria are exposed to adverse conditions and lose their ability to survive. This separation must then be regarded as a pathological phenomenon. The concept of a diffuse bacterial nucleus was already supported by N. F. Gamaleya (1940). It was later fully substantiated by A. N. Belozerskiy's biochemical investigations (1944, 1947, Trudy II Sessii Otdeleniya Mediko-Biol. Nauk, AMN SSSR, Works of the Second Session, Department of Medical and Biological Sciences, Academy of Medical Sciences USSR, 1949; XII Vsesoyuz S'ezd Gigien., Epid., Mikrob., i Infekts., II, pp 85-87, 1949-A). This investigator (1939, 1949) also demonstrated that bacteria contain an exceptionally high proportion of nucleoproteids and nucleic acids, a condition which explains their superior vitality and adaptability.

The fact that bacterial nuclei are diffuse was confirmed by electron-microscopic investigations (B. I. Isayev, A. Ye. Kriss, Ye. A. Rupkina, 1945; V. I. Biryuzova, 1949).

Experiments on the transformation of nonvirulent Type II pneumococci into virulent pneumococci of Type III show that this transformation is produced by deoxyribonucleic acid derived from Type III.

Transformation of one type into another is observed not only in pneumococci. Gracheva (1946), by cultivating *B. coli* in a medium containing killed paratyphoid (Breslau) microbes, isolated bacteria which resembled Breslau bacilli very closely. The new properties acquired by the bacilli proved to be inheritable. Gracheva's result is in agreement with earlier data obtained by L. A. Zil'ber (1929) on the paraimmunity of bacteria (Zil'ber and Korshunova, 1949). When *B. coli* are cultivated together with typhoid bacilli, they acquire the antigenic properties of the latter and bring about formation of antibodies that are active against typhoid. *B. proteus* can be modified to resemble antigenically the causative factor of typhus. Again, the newly acquired properties are inherited for a long time in this case.

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V. BACTERIAL POLYSACCHARIDES

The presence of a complex polysaccharide which causes type specificity is not a characteristic property exhibited by microorganisms alone. Higher animals also show division into immunologically distinct groups within the same species. A. Stepanov, A. Kuzin, Z. Makayeva, and P. Kosyakov (1940) showed that human beings fall within specific blood groups by reason of the specificity of polysaccharides which enter into the composition of their blood. Complex specific polysaccharides that are analogous to polysaccharides of S-bacteria were isolated from the organs of human beings, guinea pigs, and white mice (A. M. Kuzin, I. S. Buyanovskaya, A. M. Rykaleva, and N. I. Kuzina, 1947), from equine plasma (V. M. Krasov, 1947), from some helminths (S. N. Babadzhanyan, Zhur. Med. Parazit., Vol IV, pp 34-38, 1947; A. M. Kuzin S. N. Babadzhanyan, and O. I. Polyakova, 1949), and from malignant tumors (A. M. Kuzin, N. I. Kuzina, 1949).

Methods of isolating bacterial polysaccharides have been developed by A. M. Kuzin (1940; 1946) and V. I. Tovarnitskiy (Zhur. Mikrobiol., Epidemiol., i Immunobiol., No 2, pp 37-47, 1947).

Of particular interest are the glucosamines contained in specific bacterial carbohydrates. Many bacteria which are pathogenic to humans contain glucosamines in their polysaccharides. This includes Shiga's dysentery bacilli, *Str. pyogenes*, *R. prowazekii*, and pneumococci of Type I and Type IV. *Proteus X₁₉*, which is closely related to the causative factor of typhus and has the capacity of living in the human organism, is also distinguished by a high content of glucosamine in its specific polysaccharides (A. Kuzin, N. Nevrayeva, 1945). A comparison of the chemical composition of polysaccharides of mouse typhus [*Typhoid?*] with those of human typhoid shows that the latter contain 6% of glucosamine, while the polysaccharides of bacteria causing the mouse disease (bacteria which are not pathogenic to humans) lack glucosamine entirely. Of particular significance in that connection becomes the work of A. Stepanov, A. Kuzin, Z. Makayeva, and P. Kosyakov (1940), who established that glucosamine is present in the specific human carbohydrates which determine to what blood group an individual belongs.

It would seem that correspondence between the chemical composition of carbohydrates of the microorganism and those of the parasite is of importance in determining the virulence of the parasite. This assumption becomes plausible in the light of V. I. Ioffe's finding (V. I. Ioffe and K. M. Rosental', Zhur. Mikrobiol., Epidemiol., i Immunobiol., No 3, pp 23-35, 1944) to the effect that microbes which have adapted themselves to parasitism in the body of some animal, acquire the property of being agglutinated by the antiserum which is specific for the organs and tissues of the host. Thus, the scarlet fever streptococcus gives a strong agglutination reaction with antihuman serum in the first period of the disease and ceases to give it during the period of convalescence. The plague bacillus, which is so virulent to humans, has, according to N. Zhukov-Verezhnikov and G. Guseva's data (Zhur. Mikrobiol., Epidemiol., i Immunobiol., No 3, pp 14-16, 1944) a common antigen with human erythrocytes.

VI. LIPIDS OF BACTERIA

Ye. M. Gubarev (1946) demonstrated that the most characteristic fatty acid of diphtheria bacilli is hydroxydiphtheric acid, which has a hydroxyl group and an unsaturated bond. Gubarev and I. L. Vakulenko (1945) also showed that the wax fractions of diphtheria bacilli contain a high-molecular unsaturated alcohol, which was named by them corinic alcohol. According to Gubarev and Ye. K. Lubents (1948), carbohydrates also enter into the composition of the lipids of diphtheria bacilli. They are represented here by a nonreducing galactose

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disaccharide, the hydroxyl groups of which are completely esterified with fatty acid residues and also by a small quantity of pentoses. Esters of carbohydrates with higher fatty acids were named "microsids" [mikrozidy] by Cubarev.

Steroids are completely absent from the lipids of bacteria. Furthermore, addition to nutrient media of salts of bile acids sharply suppresses the propagation of most bacteria. This phenomenon was utilized to breed dysentery bacilli which are less sensitive to the action of bile acids. Use of a nutrient medium containing salts of bile acids (Ploskirev's "Zh" bacterial agar) increased the percentage of successful seeding out by a factor of two in comparison with ordinary differential media (Libinson, 1949; Zheleznova, 1950).

VII. COMPLETE BACTERIAL ANTIGEN

The problem of the complete bacterial antigen, in addition to its theoretical significance, is of considerable practical importance. Complete bacterial antigens purified from immunologically inactive admixtures, which are often harmful to the organism of humans or animals, can be used for prophylactic inoculations. Complete antigens which have been isolated were identical with bacterial endotoxins. The first successful experiments on the isolation of complete bacterial antigens in a purified form were carried out in 1928 by V. A. Krestovnikova, Ye. M. Ryakhina, and Ye. B. Ginzburg (Zhur Mikrobiol., Epidemiol., i Immunobiol., No 7 II, p 36, 1933; No 12, 401, 1934; No 15, 103, 1935).

Complete bacterial antigens were isolated from many bacteria: representatives of the intestinal-typhoid-dysentery group, cholera and pseudocholera vibrios, meningococci, brucellosis bacilli, plague bacilli, etc. Complete antigens were also isolated by USSR investigators from the organs and bodies of higher animals, including humans; the occurrence of specific substances of this type is by no means limited to bacteria. So far, complete antigens could not be isolated from gram-positive microorganisms or bacteria of the R-form.

Originally, the production of full-valued antigenic preparations from typhoid-paratyphoid bacteria (to be used for diagnostic purposes) was launched at the Moscow City Bacteriological Institute (N. G. Sheyman, Zhur. Mikrobiol., Epidemiol., i Immunobiol., No 5, pp 3-8, 1940). Aleksandrov and Gefen used complete bacterial antigen for the preparation of polyvalent vaccines, the production of which was first started at the Moscow Institute imeni I. I. Mechnikov (N. I. Aleksandrov, XII Vsesoyuz. S'ezd Gigien., Epid., Mikrob., i Infekts., No 17 II, pp 80-85, 1949).

Other methods for extracting complete antigens besides those proposed by A. Boivin, N. Raistrick, and W. Topley were developed. V. A. Vilenskiy, in Sol'vatatsiya Belkov v Dvukomponentnykh Rastvoritelyakh (Solvation of Proteins in Two-Component Solvents), dissertation, Moscow, pp 256-278, 1947, described how antigens can be extracted from bacteria with organic solvents which are nonelectrolytic and promote solvation and solution. Specifically, antigens can be extracted from bacteria with the aid of diethylene glycol, urea, phenol (A. P. Konikov, V. A. Zhidkova, Zhur. Mikrobiol., Epidemiol., i Immunobiol., No 1-2, pp 108, 1942), essential oils, formamide, pyridine (A. M. Rykaleva, V. S. Gostev, Zhur. Mikrobiol., Epidemiol., i Immunobiol., No 7, p 30, 1942), dioxane, ether, and concentrated sugar solutions (A. M. Kuzin, O. I. Polyakova, Zhur. Mikrobiol., Epidemiol., i Immunobiol., II, pp 52-56, 1947). A. M. Kuzin and S. I. Chernyakhov (Zhur. Mikrobiol., Epidemiol., i Immunobiol., II, pp 52-56, 1947) developed a method of extracting the complete antigen by extraction with hydrochloric acid, followed by removal of free proteins by adsorption.

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Using ultrasound, S. A. Blinkin, A. P. Gorodina, and I. G. Polotskiy (Zhur. Mikrobiol., Epidemiol., i Immunobiol., No 5, p 72, 1946) succeeded in extracting the antigen from the cells of dysentery microbes, while I. Ye. El'piner and A. P. Sheynker (1946), using the same method, isolated the antigen from whooping cough bacilli. V. S. Gostev and A. M. Rykaleva (Vopr. Med. Khim., No 2, 1950) established that the best extracting substances for antigens, in the order of decreasing activity, are urea, pyridine, phenol, and formamide.

The first investigators who isolated complete antigens (including Krestovnikova and Ginzburg) held that substances of this type do not contain protein. N. N. Zhukov-Verezhnikov (Zhur. Mikrobiol., Epidemiol., i Immunobiol., No 4, p 43, 1939), among others, disagreed with that view. At present, it is established that the specific carbohydrate is a hapten (prosthetic group), and that complete antigenic activity is exerted only when the hapten is attached to a protein moiety. The phospholipid part of the antigen, which is bound to the carbohydrate and protein parts, possesses neither antigenic properties nor specificity as such. Nevertheless, it has an important biological function. In the case of gram-negative bacteria, the phospholipids of their complete antigens apparently confer relative stability toward antiseptics.

On the basis of his experiments, A. N. Belozerskiy (1949) came to the conclusion that the complete antigen, combined with nucleoproteids, represents the apparatus for the reproduction of the complete antigen, which is located on the surface of the bacterial cell. When bacteria change from the S-form to the R-form, they not only lose the antigen disposed on the surface, but also the apparatus which reproduces the antigen, i.e., the complete antigen combined with the nuclear elements of the cell.

VIII. PHYSICO-CHEMICAL PROPERTIES OF BACTERIA

The study of regularities in the changes of the chemical composition of bacteria as a basis of their living processes is at the very beginning. A transition from the static method of investigating the chemical nature of bacteria to the dynamic is the main task of contemporary microbiological chemistry. The dynamic method of investigation was initiated quite recently by USSR scientists; cytological investigation of cultures was carried out in connection with the work in question (M. A. Peshkov, A. N. Belozerskiy, A. M. Korneyeva, and V. G. Karpukhina, 1947). The investigators who did this work found that bacterial cells, after 5 hr of growth, can be deeply stained with basic dyestuffs; they contain two to three times more nucleic acids than cells of the same cultures which have been grown for 20-48 hr. In other words, the older cells have become less basophilic.

In young cells, nucleic acids are firmly bound to proteins. As the age of the culture increases, the bond of these acids to proteins becomes more labile (Belozerskiy, 1941).

The results obtained by USSR scientists were confirmed by the Swedish biochemists B. Malmgren and C. Heden (1947), who used ultraviolet spectrophotometry in studying the nucleic acids in bacteria.

IX. THEORY OF STAINING OF BACTERIA

The fact that not only bacteria, but also virus proteins lose their gram-positive properties after being extracted with bile acid salts was established by L. M. Tarasevich (1947).

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Transitory states between gram-negative and gram-positive forms are observed not only in the phylogenesis of bacteria but also in their ontogenesis. Young, growing cells of gram-negative bacteria resemble in many respects gram-positive microorganisms. They are rich in nucleoproteids, which increases their acidity and basophilic properties. This makes them sensitive to the bactericidal action of basic dyestuffs, as well as to the action of various antibiotics. In some cases, normally gram-negative bacteria acquire the property of being positively stained, according to Gram, during the stage of active multiplication. Typically gram-positive bacteria stain particularly well at an early age. On the other hand, old, dying cultures of typically gram-positive bacteria acquire properties which bring them nearer to gram-negative microorganisms as far as their fundamental characteristics are concerned.

Addition of small quantities of a phospholipid (cephalin) to gram-positive bacteria effectively protects them against the antibacterial action of gramicidin in the test tube, as well as in animal experiments. On the other hand, addition of basic proteins (protamine and histone) to gram-negative bacteria makes these bacteria sensitive to antibiotics which ordinarily do not act on them. The reason is that basic proteins precipitate phospholipids of the cephalin type.

X. VITAMINS OF BACTERIA

Nicotinic acid, or its amide, are of particular importance for paratyphoid and dysentery bacteria. This acid is the only growth substance required by them. When the microorganisms in question are grown on media of simple composition, they are unable to synthesize the pyridine ring independently. A. M. Kuzin and R. Yudelovich (Zhur Mikrobiol, Epidemiol, i Immunobiol, No 12, p 64, 1944) demonstrated that ordinary meat-peptone media contain an adequate quantity of nicotinic acid. If the nutrient medium has a simpler composition, the growth of paratyphoid and dysentery cultures depends to a considerable extent on the addition of nicotinic acid. The important role of pyridoxine (Vitamin B₆) in protein metabolism is connected with its ability to add reversibly hydrogen and an amino group.

Pyridoxine is contained in decarboxylases which catalyze the decarboxylation of important amino acids such as tyrosine, lysine, glutamic acid, and arginine. Furthermore, pyridoxine enters into the composition of aminophosphatases, i.e., enzymes which bring about the transfer of amino groups from amino acids to keto acids, thus forming amino acids of another type. The reaction of transamination which is involved here was discovered by the USSR scientists A. Ye. Braunshteyn and M. G. Kritsman. The specificity of decarboxylases and aminophosphatases of amino acids, with respect to the substrate on which they act, varies widely. The differences among enzymes of this type are limited to the composition of the active carrier proteins: they all contain the same prosthetic group, namely pyridoxal phosphate (cf A. Ye. Braunshteyn, "Biokhimiya Aminokislogo Obmena" /Biochemistry of Amino Acids Metabolism/, 1949). Some bacteria are unable to synthesize pyridoxine, which is essential for the synthesis of amino acids entering into the composition of their proteins: they require a supply of pyridoxine, unless the necessary amino acids are contained in the nutrient medium.

Addition of avidin to a culture of bacteria which are unable to synthesize biotin suppresses their development, but avidin has no effect on the propagation of microorganisms that are capable of synthesizing biotin, as, for instance, B. coli. The quantitative nature of the reaction between biotin and avidin

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(avidin reacts with the urea group of biotin, forming a complex which is inactive and cannot be assimilated) enabled N. D. Yerusalimskiy (1947) to develop a method of determining biotin by means of biological titration with avidin. Acetone-ethyl ~~alcohol~~ bacteria, which are unable to develop in the absence of free biotin, are used as an indicator.

Since a very small quantity of a vitamin is sufficient to induce growth of a microorganism, the growth of a bacteria is a very sensitive indicator of the presence of a vitamin (Ye. N. Odintsova, 1949). Bacteria are such sensitive and specific indicators of vitamins that they may serve to detect the presence of the latter in the air. M. N. Meisel' and G. A. Medvedeva (1947) have shown that vitamin B₁, paraaminobenzoic acid, and nicotinic acid are volatile. Microorganisms are capable of assimilating these vitamins from the air, synthesizing the corresponding enzymes from them.

Resistance to sulfonamides is the result of an increased capacity of bacteria to synthesize paraaminobenzoic acid. As shown by Troitskiy and Pershina (1949), resistance of this type to the action of a chemotherapeutic agent is an acquired characteristic which is inherited by a great number of generations.

The competition of vitamins with their chemical analogs seemed to be a promising basis for the synthesis of other chemotherapeutic agents besides sulfonamides. However, up to date, it has not yet been possible to develop additional drugs by synthesizing vitamin antagonists. One of the reasons is that the vitamins with which vitamin antagonists compete are essential not only for bacteria, but also for the animal infected by them.

The theory in regard to the competition of various substance in the organism, which has been proposed by US investigators, does not stand trial by experiments and practical experience, since it is too mechanistic. A critical review of the theory of biological competition in the light of Michurin's theory is an urgent task of Soviet microbiological chemistry and chemotherapy.

XI. CARBOHYDRATE METABOLISM OF BACTERIA

Phosphorolysis of carbohydrates is apparently a widespread phenomenon in bacterial metabolism. Phosphorolysis in bacteria was originally discovered by Kagan, Lyatker, and Tsfasman (1942). These investigators showed that a culture of *Leuconostoc mesenteroides* in the presence of saccharose binds inorganic phosphate and that this does not take place in the presence of glucose and fructose. As a result of the binding of inorganic phosphate, saccharose splits under formation of glucoso-1-phosphate. Mechnikov's vibrio decomposes starch and maltose by phosphorolysis, binding inorganic phosphate and forming glucoso-1-phosphate (P. K. Tabakov, XIII Nauch Sessiya Saratov. Med. Inst, pp 30-31, 1948).

The central role of pyruvic acid in fermentation was established simultaneously and independently by S. P. Kostychev (1924) and A. F. Lebedev (1924). This discovery by two Russian investigators received general recognition only 10 years later, as a result of Embden's work (1933).

It has been established that heterotrophic microorganisms assimilate carbon dioxide. This includes *Aspergillus niger* (N. L. Rabotnova, 1950). *Br. abortus* is incapable of growing in an atmosphere which lacks carbon dioxide. On the contrary, an increased carbon dioxide pressure stimulates the growth of this microorganism, a phenomenon which has been utilized from the practical standpoint in the cultivation of brucellosis bacilli. The necessity of the presence of carbon dioxide has also been established in the case of many other heterotrophic bacteria. If the atmosphere does not contain carbon dioxide, and any carbon dioxide which may be formed by fermentation is removed, the

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growth of typhoid bacilli, *B. coli*, *B. anthracis*, *B. pyocyaneus*, diphtheria bacilli, tuberculosis bacilli, *Staph. aureus*, anaerobes, fungi, and protozoa is inhibited (T. F. Aristovskaya, 1941). Even a respiratory activity like the reduction of methylene blue by bacteria in a state of rest depends on the presence of carbon dioxide.

The difference between autotrophic, photosynthesizing organisms and heterotrophic organisms which do not photosynthesize is not that the first require carbon dioxide, while the latter do not. Discovery of the fact that all living beings, including bacteria, require carbon dioxide, that heterotrophic organisms assimilate it, and of the extraordinary physiological significance of this assimilation represent one of the most outstanding achievements of biological thinking during the present century. The light energy consumed by photosynthesizing organisms is not needed, as was assumed formerly, to reduce carbon dioxide to formaldehyde: the initial product of photosynthesis, in the light of contemporary data, is in all probability a triose containing an asymmetric carbon atom. It is precisely for the creation of optical asymmetric, which is a universal property of living matter, that the energy of the sun is used in photosynthesizing organisms: heterotrophic organisms which do not synthesize optically active compounds require a supply of optically asymmetric compounds that are analogous in their configuration to levorotatory lactic acid. The basis for this understanding of the role of optical asymmetric was laid by V. I. Vernadskiy (1931, 1939), who was the first to establish a close connection between basic ideas of crystallography and chemistry and problems of morphology, physiology, and the fine molecular structure of living matter.

According to G. F. Gauze (1947, 1948, 1950), the mechanism of the action of antibiotics is based on the optical asymmetry of physiologically active compounds contained in bacteria. Antibiotics usually have some elements of structure that exhibit an inverted optical activity. By reason of their inverted activity and abnormal structure, antibiotics are able to combine with the enzymes of bacteria. As a result, the enzymes are inactivated and the living processes of bacteria inhibited.

V. I. Shaposhnikov and V. A. Semenova (1949) established that the character of fermentation in a bacterial culture varies with the stages of development. During the first period of bacterial growth (i.e., the logarithmic phase), which is distinguished by very rapid multiplication of bacteria, there is a predominance of products of the oxidative fission of pyruvic acid (acetic acid, ethyl alcohol, carbon dioxide). As the rate of multiplication drops and the stationary phase of development is approached, the products of reduction of pyruvic acid (i.e., lactic acid) begin to predominate in the culture medium. The reason for this change is that in the earlier stage of development proteins which are more highly reduced than carbohydrates are synthesized from the latter to a much greater extent than in the subsequent stage. The reduction which takes place during this synthesis is counterbalanced by oxidative processes. In the stationary phase, no hydrogen is used up: the ratio between oxidation and reduction products gradually diminishes, approaching unity. The substance which is then formed from carbohydrates, as a result of a balanced consumption of hydrogen and oxygen is lactic acid.

XII. PROTEIN METABOLISM OF BACTERIA

In experiments with heavy hydrogen, USSR scientists demonstrated that the rate of synthesis and decomposition of proteins varies not only depending on the organ in which the proteins occur but also with regard to individual protein fractions of the same tissue or organ (A. S. Konikova, II Sessiya Otd. Med. Biol. Nauk, Akad Med Nauk SSSR; Problema Belka p 25, 1949).

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During the Soviet period, A. V. Blagoveshchenskiy and G. V. Eremeyev demonstrated that Danilevskiy's plasteins (1895) actually form as a result of the synthesizing action of proteolytic enzymes, because plasteins differ very little in their composition from authentic protein. This was confirmed by I. G. Menzorov (1939), who established that plasteins are not split by peptidases or dipeptidases but only by proteinases (pepsin and trypsin), which act on the complete molecule of the protein. In this manner, it could be shown that in the case of plasteins, we are actually dealing with products of the resynthesis of proteins from the peptones into which these proteins were originally split as a result of the action of pepsin (cf. A. V. Blagoveshchenskiy, *Fermenty [Enzymes]*, pp 117-120, 1940).

The reversibility of the action of proteolytic and amylolytic enzymes was shown in S. Ye. Bresler's experiments (S. Ye. Bresler, G. V. Samsonov, and N. A. Seleznev, 1949). In these experiments, it was demonstrated that increasing the pressure to 5,000-10,000 atm displaces the equilibrium due to the action of enzymes from hydrolytic splitting of proteins and carbohydrate polymers towards their synthesis. After enzymatic hydrolysis of serum albumins, the products of their proteolysis have a molecular weight of 800-900; there is no measurable quantity of high-molecular proteins in a solution of these products. However, when a protein which has been hydrolyzed in this manner is subjected to the action of a proteolytic enzyme, and high pressure is applied at the same time, resynthesis of the protein takes place. Although the products of resynthesis are not uniform, a fraction having a molecular weight of 70,000 predominates. This value corresponds closely to the molecular weight of the initial protein. The antigenic activity, which disappears on hydrolysis, is restored as a result of the resynthesis under pressure. The immunogenic properties of the resynthesized protein are exactly the same as those of the initial protein.

According to A. I. Oparin's theory (1937), which has been confirmed by many experimental facts, the enzymes of a cell are easily adsorbed by protein structures. When adsorption of this type takes place, the hydrolyzing capacity of the enzymes drops, while the synthesizing capacity is sharply increased. The direction in which the enzyme action in cells is exerted (i.e., whether synthesis or hydrolysis takes place) depends on the ratio of adsorbed enzyme to free enzyme (A. L. Kursanov, 1940). Due to surface tension, the condition which exists in the surface layer where the enzyme is adsorbed corresponds to one of increased pressure, so that synthesis is stimulated in the same manner as in Bresler's experiments.

The physiological state of the bacterial cell determines the direction in which biological synthesis proceeds and the nature of the products which are synthesized. For instance, formation of streptolysin by a streptococcus culture which is at rest does not take place when the microorganism is at a mature age, even though all substances that are necessary for the formation of this toxin (ribonucleic acid, fermentable carbohydrate, phosphates, and other salts, etc.) may be present in the nutrient medium. However, synthesis of streptolysin will take place actively under the same conditions if the culture is in the logarithmic stage of growth.

An increased sensitivity of the cell to the action of specific substances is a necessary prerequisite for an increased synthesis of cell proteins. The increased sensitivity of vigorously multiplying cells is connected with a raised physiological activity of enzymes, i.e., with a higher intensity of cell metabolism. The biosynthesis of all cells, including bacteria, is sensitive not only to proteins which are specific for their own species: foreign specific proteins may also influence the course of living processes.

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XIII. RESPIRATION OF BACTERIA

The classification of bacteria according to the four fundamental types of respiration (strictly anaerobic bacteria, strictly aerobic bacteria, microaerophils, facultative anaerobes) is arbitrary to a certain extent. First of all, the effect of the environment may substantially alter the type of respiration. As shown by M. M. Levitov, Ye. D. Vyshepan, and A. Nenashev (1946), the pseudocholera vibrio behaves like an obligatory anaerobe in a meat-peptone medium that does not contain glucose. It forms a film on the surface of the nutrient medium, leaving the lower layers of the liquid clear. If the vibrio is cultivated in a meat-peptone medium to which glucose had been added, the character of its growth changes radically. A surface film is no longer formed: the culture forms a uniform turbidity throughout the whole depth of the nutrient medium. This is a result of a weakening or complete suppression of the respiratory activity, i.e., the resorption of oxygen. In other words, the obligatory aerobe changes into a facultative anaerobe. Similarly, anaerobic microorganisms do not necessarily require an absence of oxygen: they may grow in the presence of air if substances which have reducing properties (e.g., sterile tissues, cystein thiolactic acid) are added to the nutrient.

Curves indicating changes in the redox potential of bacterial cultures are highly characteristic: they may be used in distinguishing between closely related species, in measuring the action of a bacteriophage, etc. The connection between the redox potential of the nutrient medium and the type of metabolism of microorganisms was investigated by a number of USSR scientists (E. S. Kanel', 1935; N. N. Krasina, 1936; N. D. Yerusalimskiy and M. N. Bekhtereva, 1936; N. L. Rabotnova, 1941; V. I. Lyubimov, 1950). In formulating a nutrient medium, one must take into consideration not only its p_H , but also its r_H . The r_H is by no means without influence on the optimal growth of aerobic bacteria (E. S. Kanel', 1937).

As shown by S. S. Elizandrova and M. N. Meysel' (1949), catalase activity may serve as an indicator of a normal or pathological condition of the microorganism. According to N. D. Anina-Radchenko (Zhur. Mikrobiol., Epidemiol., i Immunobiol., pp 7-8, 105-111, 1939), the living capacity of a microorganism and its catalase activity depend on each other in a regular manner.

The redox potential curve is different, depending on the presence or absence of catalase in bacteria.

In view of the fact that true dysentery bacilli do not decompose hydrogen peroxide, while pseudodysentery bacilli possess catalase activity, it was proposed to use the catalase reaction to diagnose dysentery bacilli which may be present together with other members of the group. Gartokh, Rapoport, and Linnikova (1925), Dauman (1927), and Troitskiy, Bam, and Shiril' (1929) found that all strains of Shiga bacilli lack catalase, while Flexner bacilli, which ferment mannitol, always exhibit catalase activity to a greater or smaller degree. One may diagnose Shiga bacilli without fail by placing drops of hydrogen peroxide on individual transparent colonies in the dishes containing nutrient medium.

The importance of catalase for bacteria induced US researchers to advance the anticatalase theory to explain the action of sulfonamide drugs. This theory was disproved by G. N. Pershin (Zhur Mikrobiol., Epidemiol., i Immunobiol., No 2, pp 83-86, 1947), whose experimental results show that sulfonamide drugs do not affect the activity of catalase. M. M. Zabolotskaya established that neither the antibacterial action of chemotherapeutic agents nor the bactericidal effect of disinfectant can be explained by the inactivation of bacterial catalase. The concentration of a disinfectant which sharply suppresses catalase activity is always considerably higher than the minimal concentration exerting

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a bactericidal effect (cf., M. M. Zabolotskaya, Vliyaniye Dezinfitsiruyushch i Khimioterapevticheskikh Veshchestv na Katalaznuyu Aktivnost' Bakteriy /The Effect of Disinfecting and Chemotherapeutic Substances of the Catalase Activity of Bacteria/, dissertation, Moscow, 1949).

Pershin (1947, 1948, 1949) found that disinfectants suppress the action of bacterial enzymes to a different extent depending on the nature of the enzyme (cf., G. N. Pershin, Vliyaniye Dezinfitsiruyushchikh i Khimioterapevticheskikh Veshchestv na Bakterial'nyye Fermenty /The Effect of Disinfecting and Chemotherapeutic Substances on Bacterial Enzymes/, dissertation, 1949). As distinguished from iron-containing oxidases, dehydrogenases are very sensitive to the action of disinfectants. Furthermore, in the case of any substance, the concentration which produces a distinct bactericidal effect coincides with the concentration that impairs dehydrogenase activity. This proves that the bactericidal action of disinfectants is due to impairment of the dehydrogenase activity of bacteria. The bactericidal effect of elevated temperatures is due to the same cause: there is an exact parallel between inactivation of dehydrogenases and the bactericidal effect produced by a high temperature. The same thing applies to the action of traces of heavy metals and to any other exterior influences which kill bacteria. The manner in which dehydrogenases are affected by bactericidal agents depends on the chemical composition and physicochemical properties of the latter.

The catalase theory was also used to explain the mechanism of anaerobiosis. The assumption was made that oxygen is toxic to anaerobic bacteria, because bacteria of this type form hydrogen peroxide from oxygen. Not having any catalase, the bacteria are then unable to decompose the hydrogen peroxide, with the result that they are killed by it. However, formation of hydrogen peroxide by anaerobic bacteria could not be proven experimentally. Furthermore, oxygen is not toxic to many microorganisms which lack catalase.

A group of USSR investigators headed by V. A. Engel'gardt (1944) proposed another explanation of the toxic effect exerted by oxygen on anaerobic bacteria. Engel'gardt explains the sensitivity of anaerobes to oxygen in the following manner. In the presence of oxygen, the Pasteur effect takes place in anaerobic microorganisms, i.e., there is inhibition of fermentation brought about by impeded conversion of hexose monophosphate into lactic acid. This kills the anaerobic microbe, because elimination of fermentation processes is not compensated for by oxidative fission of carbohydrates: anaerobes lack the respiratory mechanism which is necessary for that.

Subsequently to O. Warburg's work on the subject (1939), the role of phosphoric acid in respiration was studied in greater detail by Engel'gardt. Phosphoric acid is capable of entering into compounds which, as a result, store considerable amounts of energy. Energy-rich compounds of this type and the energy-rich bonds in these compounds were called macroergic or macroergs by Engel'gardt and M. N. Lyubimova. The mechanism of biological utilization of the energy stored in macroergic phosphates was originally clarified in experiments carried out by Engel'gardt and Lyubimova (1939, 1941, 1942). They showed that myosin in muscles is capable of reacting with adenosinetriphosphoric acid. The latter then decomposes under evolution of energy. This energy brings about contraction of myosin (muscle) fibers.

The theory of macroergic compounds is applicable in its entirety to bacteriology. In the light of this theory, the fact that streptolysin S is developed by a streptococcus culture only on addition to the medium of a salt of phosphoric acid and of fermentable sugar becomes understandable. As a result of phosphorylation that is tied up with fermentation, a readily available store of energy is accumulated. Without this energy, the microorganism is unable to synthesize the toxin.

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CONCLUSION

The basic idea of Michurinist biology is that of the leading role played by metabolism. Michurin and Lysenko hold that changes in the type of assimilation, i.e., in the type of metabolism, determine changes in heredity. The new methods of selection developed by Michurin, which involve extensive use of vegetative hybridization, represent a practical realization, as well as convincing proof of this idea.

At present, the trend in USSR bacteriology is toward acquiring mastery of biochemical methods, toward biochemical interpretation of living processes in bacterial cells, and in the direction of understanding the chemical activity of bacteria from the biological standpoint. This trend originated in consequence of the fact that the level of USSR microbiology has been lifted higher in all fields in agreement with prevalent tendencies dominating social and scientific national thought. The building of Communism sets before microbiologists the task of completely eliminating all disease and excluding every unhealthy condition from human life. This huge task can only be accomplished if the work is carried out on a high theoretical level. The reactionary aspects of Weisman's, Morgan's, and Virchow's theories must be eradicated with that end in view.

O. B. Lepeshinskaya's discovery of the noncellular origin of cells (1945), which is in complete accordance with Michurin's and Lysenko's teachings, represents a considerable advance along the path that has been indicated. Many discoveries by Soviet scientists, who established that there are invisible, i.e., noncellular stages in the course of development of bacteria, become understandable in the light of Lepeshinskaya's findings. The work of N. N. Zhukov-Verezhnikov, Timakov, M. D. Utenkov (*Mikrogenerirovaniye* [*Microgeneration*], 1941), G. Kalina (*Izmenchivost' Patogennykh Mikroorganizmov* [*Modifiability of Pathogenic Microorganisms*], 1949), V. A. Krestovnikova (*Zhur. Mikrobiol. Epidemiol., i Immunobiol.*, 11, p 36, 1933; No 12 p 401, 1934), Novogrudskiy, and G. M. Bosh'yan deals with the subject in question.

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